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THE GRAM-NEGATIVE COCCI IN "COLDS" AND INFLUENZA *

INFLUENZA STUDIES VII

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No affection is more common in temperate zones, probably, than that group of conditions, varying largely in clinical type and manifestations, which are classed together as "common colds." Relatively little investigation has, however, been directed toward the determination of their etiology. Their usual mild course and the absence of a fatal termination doubtless account for this fact. The tremendous economic loss caused by the incapacitation for work, from time to time, of a considerable proportion of the population, gives them an importance not always recognized. The reduced resistance following colds is likewise important in that it often predisposes to more severe infections, not alone confined to the respiratory tract. It would seem desirable to obtain more exact knowledge of the cause and mode of spread of common colds and influenza.

The varied flora of the upper respiratory tract, under both normal and pathologic conditions makes the problem of the bacteriologic investigation a most complicated one. Recent studies by Bloomfield[1] have contributed materially to our knowledge of the bacteria which are normally present in the throat, and to the conditions which govern invasion by organisms not commonly present. Some circumstance other than the mere presence of a given bacterium seems to be necessary for the initiation of disease. Large amounts of bacterial growth, from cultures of various organisms, Sarcina lutea, Staphylococcus albus, B. coli[2] B. influenzae of Pfeiffer,[3] B. mucosus capsulatus of Friedländer[4] and Streptococcus hemolyticus,[1] were smeared on the tongue, pharynx, nasal septum and into the tonsillar crypts of persons free from unusual abnormalities of the upper air passages. The various organisms disappeared as a rule within 24 hours. In no case was any demonstrable lesion or general reaction set up. The mechanical flushing action of the secretions seems undoubtedly the most important element in the disposal of bacteria introduced into the

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[1] Am. Rev. Tuberc., 1920, 4, p. 247.

[2] Bull. Johns Hopkins Hosp., 1919, 30, p. 317.

[3] Bloomfield, A. L.: Ibid., 1920, 31, p. 85.

[4] Bloomfield, A. L.: Ibid., p. 203; 1921, 32, p. 10.

mouth and nose. The saliva in many instances appears to be an unfavorable medium for bacterial growth.[5] The outstanding fact seems to be that the "normal" intact mucous membranes of the upper air passages are not only impervious to the attack of certain pathogenic bacteria, but that these organisms also fail to colonize on such surfaces. Bloomfield tentatively concludes that there are certain bacteria which constitute a true mouth flora in the sense that they live and multiply and are almost constantly present in most people and persist from day to day over considerable periods of time. Another group of organisms may be occasionally recovered from the mouth, but as a rule they are present for only short periods of time. They seem to be bacteria which are essentially transients and fail to colonize or to survive on normal mucous membranes. Occasional individuals are encountered who "carry" over considerable periods of time organisms which do not belong to the normal true mouth flora.

The micro-organisms which have been studied in greatest detail in their relation to colds and to influenza are the pneumococcus, the varieties of streptococci, and the Pfeiffer bacillus. In addition to these three major groups, there are commonly present in infections of this class, a large group of cocci which are decolorized by the Gram method. Some of these are conceded to be saprophytic inhabitants of the normal nose and throat. Others are regarded as more or less foreign to the normal mucosa and ordinarily present only in pathologic processes.

Studies of the meningococcus during periods of epidemic meningitis have furnished a wealth of information concerning the gram-negative coccal flora of the nose and throat in that infection. The first extended investigation was conducted by von Lingelsheim[6] who described various species of gram-negative cocci, including the meningococcus. Elser and Huntoon[7] report at length on the gram-negative cocci observed in epidemic meningitis. Added observations have been contributed by Dunham.[8] Certain general groups have been reported by all of these workers. The various species, then, which might be expected in such a study as the present one, would include rather rarely the meningococcus, more commonly M. catarrhalis and a large group of chromogenic cocci, and in addition certain organisms such as M. pharyngis siccus, Diplococcus crassus and diplococcus mucosus, of less frequent occurrence.

The study of this group of gram-negative cocci, particularly M. catarrhalis, in their relation to the etiology of common colds and influenza, forms the basis of the work here reported.

Two general groups of acute respiratory infection have been investigated. The first included a number of common colds of varied clinical type, occurring in an urban population. The second group was composed of cases from a recurrent epidemic of influenza, developing in the early winter months of 1920, and characterized by the same general clinical picture which was manifested in the pandemic of 1918.

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[5] Ibid., 1920, 31, p. 118.

[6] Klinisches Jahrbuch, 1906, 15, p. 373.

[7] Jour. Med. Research, 1909, 20, p. 380.

[8] Jour. Infect. Dis., Suppl. 2, 1905, p. 10.

It was decided in the preliminary experiments, to determine whether or not there was any decided increased incidence of gram-negative cocci in respiratory infections, over the occurrence in the normal throat. Furthermore, was any particular kind of gram-negative coccus found more commonly in pathologic than in normal throats? By this general procedure it was hoped to acquire a certain amount of information bearing on the rôle of M. catarrhalis and the gram-negative group in common colds and influenza.

Mere demonstration of an increased incidence of a given organism in respiratory infections can, however, simply furnish a clue to its significance. Such an organism may have been present as a normal inhabitant of the mucous membrane at the time of the developing cold, and be involved in no way in the infection. It may, although normally there, be playing an accessory rôle in the inflammation. Again, it may be present as a secondary invader to the bacterium or virus primarily causing the infection. Finally, it may be the true etiologic agent. Experiments involving a determination of the progressive bacterial changes in oncoming colds can furnish valuable information on these points.

METHODS OF STUDYING THE BACTERIAL FLORA IN UPPER RESPIRATORY INFECTIONS

Two general sources of material may be taken as available in a study of this nature. Sputum is assuredly preferred in a study of the bacterial flora of the pneumonias, and might be considered as desirable in studies of the simpler infections of the respiratory tract which present bronchial involvement. Involvement of the bronchi is, however, the exception rather than the rule in the usual type of "common cold." Even when present, the sputum raised is at best scanty and irregular. Consequently, the study of the bacteriology of all upper respiratory infections has been confined to examination of material obtained by swabbing the area of infected mucous membrane.

It seemed logical to suppose that the infecting organism in a given inflammation, whether it be a gram-negative coccus or any organism, would be present in greatest numbers on the mucous membrane first affected. Physical findings were determined by another member of the staff, Dr. W. B. Sharp, to whom I am indebted for all clinical data contained in this report. Depending on the diagnosis made, certain areas were subsequently swabbed. Early in the course of the experiments it became evident that gram-negative cocci were of extremely common occurrence in the nasopharynx. Later studies have confirmed this observation. Of the first 75 cases in which gram-negative cocci were found, 65 gave cultures from the nasopharynx. An exact record was kept of the region from which each strain of gram-negative coccus was isolated. Twenty-five cases showing gram-negative cocci, were swabbed from the nasopharynx and tonsillar regions. Nineteen of the nasopharynx cultures were positive while 20 from the posterior pharynx contained gram-negative cocci. These cultures were chiefly derived from cases of pharyngitis and tonsillitis, sometimes accompanied by bronchial involvement. Another series of cases were subjected to duplicate swabbings from the nose and nasopharynx. Of 50 cases in this group in which gram-negative cocci were demonstrated, 46 gave cultures from the nasopharynx against 30 from the nasal cultures.

The region of the nasopharynx is the chief site for localization of gram-negative cocci in the pathologic upper respiratory tract. Next to the nasopharynx, they are found with greatest frequency in the tonsillar region, with the anterior nares showing the lowest incidence On the basis of this preliminary work, a routine procedure was adopted for study of all colds. Cases diagnosed as rhinitis were swabbed from the middle fossa and floor of the nostril and from the nasopharynx while all other inflammations, those of pharynx, larynx, tonsils or combinations of these cases with bronchial infection were swabbed from the nasopharynx and from the tonsillar region. Patients with cases of influenza were likewise swabbed in the nasopharynx and pharynx. Ordinary wooden applicators with sterile cotton swabs were used for the nasal and tonsillar swabs, while the bent wire with cotton swab, as introduced by Mathers [9] was employed for obtaining nasopharyngeal material. Particular care was taken in making nasopharyngeal and tonsillar swabs to avoid salivary contamination.

Methods of Culture.—Three different mediums were employed at the beginning of the work, the vitamin-blood-pour-agar plates of Park and Williams,[10] the oleate-hemoglobin-agar of Avery [11] and a 5% sheep-blood agar, made from a veal infusion base, with 2% agar added, and the reaction adjusted to P_H 7.8. The vitamine blood medium has been suggested as a general enriching medium and especially favorable to growth of the meningococcus. It seemed a highly desirable medium for culture of all gram-negative cocci. The oleate-hemoglobin medium was developed late in the course of the recent pandemic of influenza, and has been found by numerous workers to be especially suited to the culture not alone of the Pfeiffer bacillus, but of all gram-negative organisms. Plain veal infusion blood agar has been so universally suited to cultivation of organisms from respiratory conditions that it was included as the third medium.

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[9] Ibid., 1918, 22, p. 523.

[10] Amer. Jour. Publ. Health, 1919, 9, p. 45.

[11] Jour. Am. Med. Assn., 1918, 71, p. 2050.

Careful records were kept of the results obtained from the use of each of the three mediums, during the early part of the work, that their comparative value might be determined. Not only was the incidence of the various kinds of gram-negative cocci determined, but a rough percentage estimate was made of the number of colonies developing, as compared with other groups of respiratory organisms. Of the first 75 cultures, which by one medium or another gave gram-negative cocci, it was found that growths on the oleate hemaglobin plates were positive in 66 instances. The veal infusion blood agar was almost as satisfactory, in that 55 of the cases gave positive cultures on this medium. The vitamine-blood-pour plates left much to be desired. Only 32 of 75 cases, proved by other methods to include these gram-negative organisms among their flora, were positive when cultures were made on this medium. Oleate-hemoglobin agar seems to be the medium best suited for growth of gram-negative organisms from the nose and throat, although plain blood agar is almost as satisfactory. On the basis of these results, the vitamine medium was dispensed with in later work, but cultures were still made on both blood agar and oleate-hemoglobin agar, as it was felt that the factor of using an increased number of plates would in itself tend to give higher percentage findings of the organism for which we were looking. At least 4 plates, and in the early work 6 plates, have been inoculated with material from each case studied. Swabs taken from 2 areas were rubbed over a small area on the surface of the plated medium. The inoculations were then streaked with a looped needle in "sunburst" fashion. Incubation followed at 37 C. for 24 hours. Special care was taken to maintain a high moisture content in the incubator, since study of meningococci has demonstrated the decided importance of this factor in the growth of gram-negative cocci.

The plates were examined under a dissecting microscope for the appearance of characteristic gram-negative-like colonies. These colonies were fished to blood agar slants made by the same formula as the medium employed for blood-agar plates. An average of 10 colonies were fished to slants from the plates of each case, although no attempt was made to limit the number and oftentimes more were taken.

Colonies characteristic of the gram-negative cocci are readily distinguished on blood-agar medium from other types of respiratory organisms. One kind of gram-negative colony is typically that of the meningococcus. It presents a characteristic small, transparent bluish white colony which is regular in outline and of a watery consistency. The usual catarrhalis-like colony grows somewhat more luxuriantly than that of the meningococcus. Under the microscope the colony appears darker, more compact and has rather ragged margins. It has a granular appearance, especially toward the center of the colony, is semitransparent and causes no change of the medium. A third general type of colony characteristic of gram-negative cocci is one slightly smaller than the meningococcus-like colony and readily differentiated by its consistence. The colony is firm and adherent to the surface of the medium. So firm is the colony that it can readily be lifted with the needle and turned over bodily. Diphtheroid colonies are distinguished from the gram-negative-coccus colonies, with difficulty. Determination of morphology furnishes the only sure method. Staphylococci are readily differentiated by their larger size and distinct white appearance. Colonies of streptococci and pneumococci with their attendant change in appearance of the medium furnish no difficulty, nor do the colonies of the Pfeiffer bacillus.

Few colonies other than those of gram-negative organisms develop on the oleate-hemaglobin agar. This fact, together with the growth favoring qualities which it possesses, makes it a valuable medium. Occasional colonies of diphtheroids may be confused with those of gram-negative cocci. Staphylococci are commonly present, but are distinguishable by their larger size and white appearance.

After 24 hours' incubation the blood agar slant cultures picked from plates were definitely classified as belonging to the group of gram-negative cocci by morphologic examination. The Sterling[12] modification of the gram-strain has been used throughout the work. The gentian violet stain was applied for 10 seconds, the iodine solution for 20 seconds, followed by alcohol. Ten per cent. aqueous saffranin solution, applied for 1 minute, has been used as a counter stain. With each new lot of stain prepared, control preparations were made, to insure proper differentiating value. A culture of a known meningococcus was employed as a negative control, a culture of Staphylococcus aureus as a positive control.

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[12] Monograph Rockefeller Inst. Med. Research, 1917, 7, p. 20.

Cultures distinguished on the basis of morphology and Gram reaction as members of the gram-negative coccus group were reserved for classification and further study.

Vedder's starch medium was found to be very well suited for carrying stock cultures of the various gram-negative cocci. Practically all strains encountered grew readily on this medium, especially after a few generations. A few were refractory and stock cultures in these instances were maintained on blood agar until growth could be initiated on starch agar. Freshly isolated cultures were transferred at weekly intervals for the first two or three generations, after which monthly transfers sufficed. Every third or fourth transfer of stock was put onto blood agar instead of starch agar, and after a week retransferred to starch agar. It is a matter of common experience that stock cultures of these organisms are maintained with difficulty, for occasionally a strain will die out, even after long artificial cultivation and for no apparent reason. By these methods, however, unusually good results have been obtained.

THE CLASSIFICATION OF GRAM-NEGATIVE COCCI OF THE NOSE AND THROAT

The essential purpose of this investigation has been a classification of the gram-negative cocci which occur in the normal and pathologic upper respiratory tract. Having effected a classification, the incidence of the different groups in various types of common colds and in influenza will be discussed, and an attempt made to determine their probable relation to the given infection.

Primary classification has been made on the basis of reaction toward various carbohydrates. General laboratory practice has favored the use of liquid carbohydrate mediums in the classification of bacteria by fermentation reactions. In our work, however, a solid medium has been employed, and has been found to be much more satisfactory and more easily controlled. Liquid medium is preferable in a study of organisms which give fermentation with production of gas, in that roughly quantitative measurements of the gas may be made. None of the gram-negative cocci heretofore described possess this property. The only other advantage possessed by a liquid medium is the fact that the degree of acidity may be measured in a broth medium by a determination of the hydrogen-ion value. This may likewise be done within rough limits for a solid medium, by preparing samples of different P_H value, and observing color values with a standard indicator. There seems then to be no decided disadvantage possessed by a solid medium in a study of this particular group, and its advantages are material. Many of the gram-negative cocci grow with difficulty in broth media. On solid medium the growth is much more satisfactory. Growth in broth can often only be determined by stains and morphological examination. On solid medium growth is readily determined. A large proportion of these organisms, furthermore, do not produce acid from any carbohydrate, and in liquid medium, especially if the broth be somewhat turbid, morphologic examination must frequently be made to insure conclusive readings. Probably the most distinct advantage possessed by an agar-base medium is the control furnished of purity of growth of the inoculated strain, and freedom from contamination during the technical procedure of transferring. Growth characteristics readily demonstrate contaminations on solid medium. Only examination of the morphology will serve definitely to control broth cultures. For these reasons, it was felt that use of medium involving an agar base would not only facilitate technical operations, but would also guard against erroneous results.

The carbohydrate mediums have been made according to the following formula: One pound of finely chopped lean veal is infused with 1 liter of distilled water for 24 hours on ice. At the end of this period the liquid portion is removed by use of a meat press and the albuminoid substances coagulated by bringing to the boiling point. The infusion is then filtered, and the total volume brought up to 1 liter. To this veal infusion 0.5 % sodium chloride, 1 % peptone and 2 % agar are added and the substances brought into suspension. The reaction is adjusted to P_H 7.8 and 1 % Andrade solution[13] added as an indicator. Sugar solutions previously prepared in distilled water were added to give a final concentration of 1 %. The medium was then steamed for 3 successive days in the Arnold sterilizer. It was then slanted, care being taken to leave at least an inch of solid medium in the butt of the tube below the sloped surface. The medium was incubated for 24 hours at 37 C. for sterility. Each lot of medium was inoculated with a known fermenting organism to insure proper reaction.

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[13] Jour. Infect. Dis., 1914, 15, p. 227.

Both stab and streak inoculations were made on the several carbohydrate mediums. It is well recognized that growth of certain of the gram-negative cocci is enhanced by a partial reduction in oxygen tension, and conditions best favoring growth were found as a rule to prevail at the butt of the tube, although surface growths were uniformly good. In some few instances growth could not be initiated, but it was found that by smearing the surface of the agar with a small amount of sterile, inactivated sheep serum that growth could always be obtained. This technic was employed for such strains.

Purity of growth in cultures was insured by making pour plates of all cultures just before they were tested for fermentation. Single colonies were fished from the plates to agar slants and examined for morphology. The medium was made in large amounts, and so far as possible strains were tested for acid production in considerable numbers at one time in order that comparable conditions of temperature, moisture and the same lot of medium might prevail.

Cultures were given an initial incubation of 24 hours at 37 C., and preliminary observations were recorded. Incubation for 7 days followed, when final readings were taken. Varied reactions have been obtained by making these double readings. Some organisms were found to be rapid acid formers, the reaction at the end of 7 days being no different from that taken at the end of the 24-hour period. The majority were slow acid producers, there being a negative reaction at 24 hours with a distinct positive reaction at the time of final observation. A third limited group produced a slight degree of acidity with certain of the sugars during the first 24 hours. Final readings at 7 days, however, showed a return to alkalinity.

The reactions to 8 sugars have been studied, namely, dextrose, levulose, and galactose of the monosaccharids, lactose, saccharose and maltose of the disaccharids, and mannite and dextrin among the polysaccharids. In the course of the work, the fermentive properties of about 502 strains have been determined. A number of duplicate strains were, of course, picked from the same plate during the examination of a given case, although an attempt was made to pick only colonies which showed apparent differences in growth characteristics. Likewise, certain cases have been studied over considerable lengths of time being frequently cultivated, and again similar strains were isolated from time to time. In the tabular summary of the fermentive reactions of the group, table 1, only case strains are included; that is, each strain included in the table represents a particular variety of organism occurring in a given case and does not include duplicate strains isolated at the same time or in subsequent examinations. The reaction of 266 such case strains are presented. They represent organisms present in the nose and throat of normal persons and of persons suffering from influenza and from cases of common colds.

There are, on the basis of results presented in table 1, two main groups of gram-negative cocci present in cultures from the normal nose and throat, and in acute respiratory infection. One group of organisms includes those cocci which do not attack any of the carbohydrates, the Micrococcus catarrhalis group. The second general group is characterized by its chromogenic properties and may be divided into various subgroups on the basis of fermentation reactions. In addition to these two larger groups, meningococci have been isolated in a small percentage of cases. Other organisms encountered are M. pharyngis siccus and Diplococcus crassus. Diplococcus mucosus, observed by von Lingelsheim[6] in a study of gram-negative cocci in epidemic meningitis was not encountered. In addition to the organisms classified in the preceding table, a few strains did not fall into any of the recognized groups. Two chromogenic strains produced a rather well marked red pigment in contrast to the greenish-yellow pigment produced by other chromogenic gram-negative cocci. Sugar reactions showed acid production from dextrose, levulose, saccharose, lactose and maltose.

TABLE 1

THE FERMENTATION REACTIONS OF GRAM-NEGATIVE COCCI OF THE NOSE AND THROAT

Organism	Number of Case Strains	Dextrose	Levulose	Maltose	Saccharose	Lactose	Galactose	Mannite	Dextrin
M. catarrhalis	98	—	—	—	—	—	—	—	—
M. catarrhalis Subgroup A *	5	± —	—	—	—	—	—	—	—
Meningococcus	8	+	—	+	—	—	—	—	—
M. pharyngis siccus	20	+	+	+	+	—	—	—	—
Diplococcus crassus	15	+	+	+	+	+	+	—	—
Chromogenic group 1	2	+	—	—	—	—	—	—	—
Chromogenic group 2	5	+	+	—	—	—	—	—	—
Chromogenic group 3	7	+	—	+	—	—	—	—	—
Chromogenic group 4	20	+	+	+	—	—	—	—	—
Chromogenic group 5	45	+	+	+	+	—	—	—	—
Chromogenic group 6	21	+	+	+	+	+	+	±	±

* One strain fermented maltose as well as dextrose. Agglutination for meningococcus, negative.

Key: — = no fermentation; ± — = very slight acidity in 24 hours, alkaline 7 days; + = distinctly acid; ± = variable reaction on polysaccharides, but one or the other fermented or both.

Three other strains produced an intensely yellow pigment. Of these 3 strains, 2 fermented dextrose and saccharose only, while the third fermented dextrose, levulose and saccharose. These 5 organisms are grouped together in succeeding tables as heterologous strains.

The largest single group of organisms is that group distinguished by lack of acid-producing power when grown on the carbohydrates tested. This group has been termed the Micrococcus catarrhalis group. It has been found to be representative of a group of organisms rather than a single distinctive species. While all of the 98 case strains included in the tabular summary have shown the common property of lack of fermentive power, morphologic and especially cultural studies indicate that there is a distinct difference among various strains. This fact has been noted by previous workers, notably by Elser and Huntoon [7] who distinguished 2 cultural types of M. catarrhalis. Based on strains studied in this work, the members of the group can be divided into 4 rather well marked subgroups, all of which fail to ferment carbohydrates, with possibly a fifth, designated in the table as M. catarrhalis subgroup A, which differs from the rest in its reactions toward sugars. The largest subgroup among the nonfermenting types corresponds to the classical description of M. catarrhalis given by Ghon and H. Pfeiffer.[14] The colonies appear after 24 hours as somewhat raised, opaque, white or gray-white disks, about the size of a meningococcus colony. When examined under the dissecting microscope, they are seen to have an irregular serrated margin and may be confused with colonies of certain diphtheroid organisms. The colony is typically granular, differing in this respect from the meningococcus colony which is more homogeneous. The different elements of the colony are strongly adherent to each other, and fragments may be detached with a needle. It differs in this toughness and tenacity of structure from most of the other types of gram-negative cocci. Morphologically the coccus is gram-negative and usually somewhat larger than the meningococcus. It characteristically appears in diplococcus form, although occasionally strains show numerous tetrads. Like most of the members of the group, it shows an early tendency to present degeneration forms, and even in young cultures, particularly in older ones, variations in size of different cells is readily observed. The cocci retain the Gram stain in some degree as swelling and degeneration progress. Irregularly stained preparations result, some cells being gram-negative, others, especially the giant forms, appearing gram-positive. These organisms grow readily in subculture and usually can be maintained on starch agar for long periods of time without difficulty. Occasionally certain strains exhibit a tendency to die out for no apparent reason, even after a long period of cultivation on artificial medium. Daily subcultures and use of blood medium are necessary to restore satisfactory growth. Sixty-eight of the 98 case strains studied belong to this division.

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[14] Ztschr. f. klin. Med., 1902, 44. p. 262.

The second subgroup corresponds in all respects to the preceding organism in colony structure, morphology and growth characteristics. It differs, however, in that a pale yellow pigment is formed by cultures grown on starch or plain veal-infusion agar. It was believed at first that these strains must belong to the group of chromogenic cocci so commonly found in the nose and throat, although the color of pigment formed was not exactly characteristic of the latter, which is a greenish yellow. Repeated transfers have been made to carbohydrate mediums in the course of the study of these strains, but never have they shown any indication of acid-producing power. Four such case strains have been isolated. One was obtained from a normal case, cultivated weekly for 3 months, with the coccus consistently present in all examinations.

A third subgroup, including 18 like strains, showed little cultural resemblance to the former 2. The colony differs markedly and indeed has much the appearance of a meningococcus colony, except that it is smaller. They are about one-half the size of the predominating type of M. catarrhalis colony and are flat, gray, rather than whitish gray, and translucent. Structurally, the colony is homogeneous, without granular appearance, as a rule, although slight granulation may appear toward the center. The surface is smooth and glistening while the edges are regular, in contrast to the jagged outline of the more common M. catarrhalis colony. In all respects it resembles a small, meningococcus colony. Morphologically the organism has practically the same appearance as other forms mentioned. Growth of recently isolated cultures is usually scant on primary isolation, but generally increases in amount after continued cultivation. Growth at best was irregular, however, and it was this group which gave the most trouble in maintaining stock cultures. Growth characteristics remained unchanged, however, with most of the strains, but 4 of them, after several months on artificial medium, gradually presented an appearance more nearly resembling the type of growth of the typical M. catarrhalis.

Another group of 8 strains differed a great deal from all other strains studied. The colonies were almost pinpoint in size, being extremely minute. They were transparent, glistening and possessed smoothly rounded edges. Such cultures, even when grown artificially for some time, have failed to show any change in characteristics, and maintain the same delicate growth. Cultures are initiated with great difficulty on other than blood agar, and to maintain them it has been necessary to transfer at weekly intervals, using blood medium. These strains are also distinct morphologically. Although derived from this fine delicate appearing colony, stained preparations of the coccus made after 24 hours' growth show the presence of a giant form of coccus with frequent metachromiasis. These stained preparations had all the appearance of a like preparation made from an old culture of the first type of M. catarrhalis, so much so that a series of stains were made from these cultures at early periods of growth, at 4, 6, 8 and 12 hour periods, to ascertain whether at these stages the organism might not present the usual morphology. At the 4-hour period the cocci were larger than the ordinary gram-negative coccus, but later at 8 and 12 hour periods the morphologic picture was much like that of the ordinary M. catarrhalis. Later the giant forms appeared. It would seem, then, that these strains represent a type of organism allied to the others, but undergoing more rapid transition into degenerative forms.

The last subdivision of the group consists of about 5 strains which agree exactly, in morphology and in growth characteristics, with the predominating type. They are different, in that they give rise to a slight degree of acid production on certain of the sugars—always dextose, and maltose, as well in the case of one of the strains. The degree of acidity produced is slight, just sufficient to impart a delicate pink color to the medium. This acidity is transitory, however, and readings at 48 to 72 hours show a loss of color, with 7-day readings indicating a final alkaline reaction. It was felt that minute differences in the reaction of different lots of medium might account for the slight initial acidity, but repeated tests gave like results. Since observations have been based on 7-day readings, these strains have been classed as members of the M. catarrhalis group, and are indicated in the table as "M. catarrhalis subgroup A." It is quite probable that these strains belong to the same group, but represent a distinct variety.

The group of gram-negative cocci which are classed as M. catarrhalis can then be divided into 4 definite subgroups on the basis of cultural differences and to a certain extent on morphologic variation. An additional subgroup is recognized which is characterized by a slight initial acidity with certain carbohydrates, followed by a terminal alkalinity. All have the common property of spontaneous sedimentation when suspended in salt solution or nutrient broth, although that property is less marked in members of the third subgroup described.

A number of gram-negative cocci, possessing a common property of pigment production, but differing widely in fermentive properties, constitute a group second in incidence to the M. catarrhalis group. With the exception of chromogenic group 6, all of these organisms agree morphologically, and growth characteristics are for all practical purposes the same. Slight variations in the quality of the pigment are evident in different strains, but in general the colonies when viewed by transmitted light have a greenish yellow color and are semi-opaque. Viewed by reflected light, the color of colonies on agar appears to be greenish gray. They are somewhat smaller than colonies of the M. catarrhalis group, being about 1 mm. in diameter at 24 hours. Rather distinct variations in colony appearance are evident in different strains. Some of them closely resemble that of M. catarrhalis, while others, particularly strains belonging to chromogenic group 3, are quite like a meningococcus colony. The usual colony appearance, however, is that of an irregularly circular disk with more or less jagged outlines and a varying granular structure. Most of the colonies possess the dense, compact qualities peculiar to the M. catarrhalis colony and can be broken up into numerous crumbly fragments when touched with a needle. The organisms of chromogenic group 6 differ from the rest. The organism occurs usually in pairs or tetrads, but occasionally decided clumps are observed in stained preparations. Colonies appear more opaque than those of other chromogenic cocci and are larger. The pigment is usually a pale yellow and does not possess the greenish tint common to the rest.

On the basis of sugar variations the chromogenic organisms can be divided into 6 different groups. Three small groups ferment, respectively, dextrose only, dextrose and levulose, and dextrose and maltose. A larger group ferments 3 of the carbohydrates—dextrose, levulose and maltose. The largest group, constituting about one-half of the strains having pigment producing power, ferments dextrose, levulose, maltose and saccharose. Group 6 ferments uniformly all of the monosaccharids and disaccharids employed, and always one or the other of the 2 polysaccharids, sometimes both. It is probable that members of this group, judged by their morphology, growth characteristics and the quality of the pigment produced, are in the light of the fermentation reactions to be regarded as aberrant or degenerated forms of the staphylococci. They were, however, consistently gram-negative and are classed with the other chromogens. The probable relationship of certain other types of gram-negative chromogens to the staphylococcus, particularly organisms corresponding to the chromogenic group 5 of this classification, has been pointed out by von Lingelsheim.

M. pharyngis siccus has been encountered a number of times. The colonies of this organism are readily distinguished from other types of gram-negative cocci by their decided firmness of structure. It is almost impossible to break them up with a needle, and indeed the whole colony can be lifted bodily from the surface of the medium. They are closely adherent to the medium and about the size of the M. catarrhalis colony. Morphologically the cocci occur in pairs, but are distinctly smaller than other gram-negative cocci observed.

Diplococcus crassus, described in detail by von Lingelsheim,[6] occurred in 10 cases of the series. It retains the gram stain with more tenacity than other gram-negative cocci, and usually weakly gram-positive and gram-negative members can be observed in the same preparation, especially in older cultures. The colony is small, grayish white and rather compact.

Meningococci constitute the last division in the group. The morphology and characteristics of growth are too well known to require detailed description. It will be noted that chromogenic group 3 and the meningococci have identical sugar reactions. In all cases, confirmation of meningococci has been made by agglutination with a polyvalent meningococcus serum.

Analysis of this classification of gram-negative cocci by cultural methods establishes the fact that the M. catarrhalis group has the most common incidence in cases of common colds and influenza, and in normal throats. Chromogenic cocci occur frequently, but the incidence of any particular type is not marked. The chromogens have been studied serologically by Elser and Huntoon[7] who demonstrate that organisms corresponding to our group 5, the most common chromogen in the upper respiratory tract, are immunologically different, although agreeing in fermentation. Strains corresponding to the present groups 2 and 3 were serologically the same. Further study of these organisms has not been attempted on account of the conclusive results of these workers, and because no group occurs with any decided frequency in cases of respiratory infection. It has seemed important to determine whether or not the different strains of M. catarrhalis, which have been found to occur so frequently, are representative of a single dominant type in this large percentage of colds and influenza and are capable of being distinguished from types of the same organism occurring in the normal nose and throat. For this reason a serologic study of representative strains has been undertaken.

Agglutination tests could not be used for, unfortunately, all of the strains with the exception of 8 of the cultural type 3, and 1 strain of the cultural type 1, had the undesirable quality of spontaneous sedimentation. Attempts were made to obtain stable suspensions by various means, such as variation of the salt content of the medium in which they were taken up, and by the addition of a minute amount of alkali or acid to the suspension, but without success.

The technic of complement fixation seemed to furnish the most practical method. Before undertaking a serologic classification by this technic, preliminary tests were made to determine the extent to which antibodies for M. catarrhalis could be developed in an experimental animal. Rabbits were injected intravenously with typical strains. Immunization is relatively easy for the animals will tolerate large initial doses of live organisms. Bacteriolytic experiments with immune serums, plus complement and a standardized suspension of cocci demonstrated that strongly lytic serums could be prepared. Serum for one of the agglutinable strains gave an agglutinin titer of 1:2,000.

Antigens were prepared for 15 different strains representative of the various cultural types, and cross-fixation experiments, following standardization of the antigens with polyvalent serums, have been undertaken. The results are as yet incomplete and will be reported in a later paper. Sufficient data are at hand to determine that the various strains which are alike on the basis of suar reactions are not representative of a single type of organism when judged by complement-fixation reactions. At least 3 different types can be differentiated. Whether the serologic grouping can be correlated with the 4 cultural types which have been described, cannot yet be determined.

THE GRAM-NEGATIVE COCCI IN THE NORMAL RESPIRATORY TRACT

The chief aim of this investigation has been directed toward a more exact knowledge of the relationship of these gram-negative coccal forms to acute infections of the upper respiratory tract. The work on classification was preliminary and essential to any interpretation of the significance of the organisms observed in various pathologic conditions. Definite knowledge of the incidence of the various groups of gram-negative cocci has been sought likewise in the normal nose and throat in order that a workable basis for comparison with the incidence under pathologic conditions might be available.

The normal carrier rate for the meningococcus has been the subject of extensive investigation during epidemic periods and depends largely on the extent of contact of the so-called normal population. In non-epidemic times the number of persons who carry meningococci in their upper respiratory tract varies, according to most authorities, from 3 to 6%, but in epidemic periods rises much higher. Frequent references in the literature to the presence of M. catarrhalis in disease conditions are available, dating from the original work of Ghon and Pfeiffer. No comprehensive study of the later organism in reference to its occurrence in the normal respiratory tract can be found. Arkwright[15] made nasal examinations of 15 normal subjects and found organisms which he classed as M. catarrhalis in 5 cases, or 33%. No information concerning the incidence of the other varieties of gram-negative cocci under normal conditions could be found. A series of 6 normal persons were studied by Bloomfield[16] at weekly intervals over a period of from 1 to 3 months. In practically all cultures of the series the predominating organisms were found to belong to the group of gram-negative cocci. No attempt was made to differentiate species other than the meningococcus. It seemed highly desirable to determine a normal incidence for all types, particularly a rate which would have been established by the same methods, and under the same conditions, as the subsequent study of respiratory conditions.

Various groups of normal persons have been studied at different times of the year in order to obtain an idea of the general incidence, as well as seasonal variations. One series was cultured during the spring months, April and May, a second during the summer months, June, July and Aug., while the third group was studied during Nov.,

[15] Jour. Hygiene, 1907, 7, p. 145.

[16] Bull. Johns Hopkins Hosp., 1921, 32, p. 33.

Dec., Jan., and Feb. The winter group includes cases from both 1919-20, and 1920-21. All of the subjects for study were drawn from the general student population at the university, and are probably representative of the ordinary urban community. No person in these groups had at the time of examination been knowingly exposed to colds, nor had they suffered from colds within recent date. A careful clinical examination was made before culturing to rule out possible inflammation of the respiratory mucous membrane. The results of the study of the nose and throat of normal persons are presented in table 2.

TABLE 2

THE GRAM-NEGATIVE COCCI IN THE NORMAL NOSE AND THROAT

Series	Number Examined	M. Catarrhalis	M. Pharyngis Siccus	Diplococcus Crassus	Meningococcus	Chromogenic					
						Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Spring........	35	15	1	0	0	0	0	0	1	8	4
Summer.......	47	24	7	2	1	0	0	1	5	6	2
Winter........	28	12	2	2	0	0	1	0	4	4	2
Total.......	110	51	10	4	1	0	1	1	10	18	8
Percentage incidence all cases.....	..	46	9	4	1	0	1	1	9	17	7

This summary presents the total number of subjects included in each series and indicates likewise the number from which organisms of the various groups were isolated. The group which does not possess fermentive power, M. catarrhalis, constitutes by far the most common type observed in the respiratory tract. Various types of chromogenic cocci come next in order. M. pharyngis siccus is present in 9% of persons. The most significant fact brought out by these experiments, conducted as they were at different times of the year, is the practical absence of any seasonal variation. All of the organisms, including the most common group, M. catarrhalis, show practically the same percentage incidence at all times of the year. It was expected that the summer series might show a lower incidence than that observed during the colder months when respiratory infections are more prevalent. Such did not prove to be the case.

THE GRAM-NEGATIVE COCCI IN COMMON COLDS

M. catarrhalis is the only organism among the group of gram-negative cocci, to which any importance has been ascribed by previous workers, as a possible factor in the cause of infections of the upper respiratory tract of the type of common colds.

The organism when originally observed by Seifert,[17] was found by him in the sputum and nasal secretions of persons affected by a small epidemic of infectious bronchitis. Later the organism was encountered by R. Pfeiffer[18] who obtained it in culture and gave it its name. He found it in large numbers in the bronchioles and alveoli in children with bronchopneumonia. Frosch and Kolle,[18] in subsequent studies, found it in other cases of bronchitis. The organism observed by Ritchie[19] in a case of bronchopneumonia in a child was doubtless the same organism. Numerous early investigators who give indeterminate descriptions of "gonococcus-like organisms" or "meningococcus-like organisms" as encountered in the simpler respiratory infections were probably dealing with M. catarrhalis. Ghon and H. Pfeiffer[14] made an extended study of the organism, which they found in numerous cases of acute bronchitis and bronchopneumonia. Lord[20] has observed M. catarrhalis in the sputum of about 12 patients with ordinary bronchitis. Arkwright[15] isolated this coccus from 11 of a series of 33 cases of catarrhal inflammation of the nose. Voorhies[21] and likewise Mackay[22] state that common colds are due to a number of organisms, among them M. catarrhalis. M. catarrhalis observed in most of the cases reported by these investigators has usually been associated with some other organism, such as the pneumococcus, the streptococcus, or the bacillus of Pfeiffer, although in a smaller number of cases, it has been found essentially as the only organism involved.

In the present study, investigation has not been confined to a determination of the prevalence of M. catarrhalis in acute upper respiratory infections, but an attempt has been made to determine the complete gram-negative flora. It seemed desirable to observe the degree of association of the other groups of gram-negative cocci with M. catarrhalis and with other organisms in respiratory infections, with the possibility in mind that they might exercise some symbiotic relationship, if no distinct pathogenesis. Our cases of colds have been obtained from the same general student population at the University of Chicago that furnished the subjects studied in the several normal series. No attempt has been made to select certain types of infection. All cases were examined as reported from time to time. Consequently the cases included in the "cold" series vary considerably in the clinical type of infection. A special effort was made to have cases available early in the course of the inflammation. The majority were observed within the first 24 hours, rarely after a period of more than 48 hours following the onset of symptoms.

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[17] Samml. klin. Vortr., Leipzig, No. 240, 1882.
[18] Flügge, C.: Die Mikroorganismen, 1896.
[19] Jour. Path. & Bacteriol., 1900, 7, p. 1.
[20] Centralbl. f. Bakteriol., I, O., 1903, 34, p. 641.
[21] Amer. Med., 1917, 12, p. 125.
[22] Brit. Med. Jour., 1919, 2, p. 159.

A total of 119 colds are included in the present series. A summary is presented in table 3 of the gram-negative cocci present, classified according to fermentation reactions, and subdivided according to differences in clinical diagnosis of the subject.

TABLE 3

THE GRAM-NEGATIVE COCCI IN COMMON COLDS

Type of infection	Number Cases Examined	Micrococcus Catarrhalis	M. Catarrhalis Subgroup A	M. Pharyngis siccus	Diplococcus Crassus	Meningococcus	Chromogenic Group						Heterologous
							1	2	3	4	5	6	
Acute rhinitis..	55	25	1	5	4	3	1	2	2	4	12	4	2
Acute rhinitis and bronchitis	9	5	0	1	0	0	0	0	1	1	4	0	0
Acute rhinitis and acute pharyngitis...	6	4	0	0	0	1	1	0	0	0	0	1	0
Acute rhinitis and tonsillitis	2	1	0	1	0	0	0	0	0	0	0	1	0
Acute rhinitis and sinusitis..	2	2	0	0	0	0	0	0	0	1	0	0	0
Acute pharyngitis...........	14	5	2	0	2	0	0	1	1	1	3	2	1
Acute pharyngitis and bronchitis..........	3	0	0	0	0	0	0	0	0	0	1	2	0
Acute tonsillitis............	11	5	0	0	1	0	0	1	0	1	1	1	0
Acute tonsillitis and bronchitis..........	1	0	0	0	0	0	0	0	0	0	1	0	0
Epidemic tonsillitis and pharyngitis...	16	7	0	1	0	1	0	0	0	1	5	1	1
Acute laryngitis..........	1	0	1	0	1	0	0	0	0	0	0	1	0
Total colds..	119	54	4	8	7	5	2	4	4	9	27	13	4
Percentage indence in all cases..........	...	45	3	7	6	4	2	3	3	8	23	11	3

One striking feature is evident when the results obtained from the study of the gram-negative cocci in colds are compared with the figures derived from the study of normal persons. The incidence of the various groups which have been differentiated agrees almost exactly in both instances. The frequency of M. catarrhalis in the normal and the pathologic throat is essentially identical, 46% in normal persons and 45% in persons with various types of colds. Similar agreement exists for most of the other groups.

No decided variation in the incidence of various groups can be observed in the different clinical forms of inflammation which have been studied. If one may compare those cases in which the infection was confined to the nose or pharynx with the fewer cases which presented bronchial involvement as well as inflammation of some portion of the respiratory tract higher up, it would seem that gram-negative cocci, particularly M. catarrhalis, occur less frequently in the latter type of cold.

In the course of the work, no attempt was made to determine the exact numbers of gram-negative cocci in given plate cultures in relation to members of the pneumococcus, streptococcus, staphylococcus and Pfeiffer bacillus groups. Rough plate readings were made, however. While colonies of the general gram-negative character could be observed in the majority of cultures—somewhat more than three-fourths of them—nevertheless it was uncommon to find the gram-negative flora constituting more than 10% to 30% of colonies. Occasionally plate cultures would be dominated by these organisms, sometimes constituting from 80% to 90% of all colonies.

THE GRAM-NEGATIVE COCCI IN INFLUENZA

A recurrent epidemic of influenza of short duration occurred during the early part of 1920, following the pandemic of 1918. The work which we were doing on common colds was suspended and our investigations were confined to a similar study of the incidence of gram-negative cocci in influenza. Only a relatively small number of cases could be investigated, due to the limited time that material was available. Three different groups of cases were studied, all occurring within the vicinity of Chicago. It was felt that if cases could be studied which developed in different localities where contact between groups was unlikely, that possibly the comparison afforded might permit a generalization on the bacteriologic conditions in this type of infection.

The presence of M. catarrhalis in influenzal conditions was demonstrated as early as 1890, when Kirchner[23] first reported it as being one of the organisms encountered in influenza, prevalent at that time in epidemic proportions. Subsequent to the epidemic of 1890, several instances are on record in which the organism has been found in interepidemic outbreaks of influenza-like infection. In certain instances the organism has been observed in connection with certain others of the common respiratory micro-organisms, such as the pneumococcus or the streptococcus. At other times, it has been considered by various writers as the chief organism involved in particular outbreaks. The studies of Ghon and H. Pfeiffer[14] have been referred to. In 1905, Dunn and Gordon[24] record an epidemic simulating influenza in which M. catarrhalis seemed to be the predominating bacterium. Bezançon and De Jong[25] describe an epidemic occurring the same year in Paris, with M. catarrhalis dominant in bacterial cultures.

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[23] Ztschr. f. Hyg. u. Infektionskr., 1890, 9, p. 528.

In the most recent pandemic of influenza, a great number of workers found M. catarrhalis in cases distributed throughout the country. Practically every bacteriologic study contains some reference to the organisms, although no detailed study of its relationship to influenza has been encountered. No attempt will be made to summarize the work of the past few years. The general deduction may be drawn that while M. catarrhalis is one of the more common species encountered in epidemic influenza, it rarely occupies a dominant position in the bacterial flora. Little importance has been accredited this organism as a factor in the pathogenesis of influenza. Finally, Clark and Murphy[26] have reported M. catarrhalis as the predominating organism in a recurrent epidemic occurring at the same time as the present group of cases.

TABLE 4

The Gram-Negative Cocci in Influenza

Case Series	Number Cases Examined	Micrococcus Catarrhalis	M. Catarrhalis Subgroup A	M. Pharyngis siccus	Diplococcus Crassus	Meningococcus	Chromogenic Group						Heterogenous
							1	2	3	4	5	6	
University of Chicago......	8	2	0	0	1	0	0	0	1	1	1	0	0
Great Lakes...	20	8	1	2	2	1	0	0	1	0	2	3	0
Camp Grant...	12	4	0	0	1	1	0	0	0	0	1	0	0
Total cases..	40	14	1	2	4	2	0	0	2	1	4	3	0
Percentage incidence all cases.........	...	35	3	5	10	5	0	0	5	3	10	8	0

The group of cases included in this report is derived from three sources. A number of the cases developed among the same general student population at the University of Chicago, from which we had been drawing our material for the study of common colds. A group of cases occurring at the Great Lakes Naval Training Station, Waukegan, Illinois, constitutes our second series. The third series was obtained from soldiers stationed at Camp Grant, Rockford, Ill. The results of the examinations from these three series of influenza cases are contained in table 4.

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[24] Brit. Med. Jour., 1905, 2, p. 421.

[25] Bull. et mém. Soc. méd. d. hôp., 1905, 22, p. 165.

[26] Clark, P. F. and Murphy, E. J.: Scientific Proc. Amer. Assn. Bacteriologists, Abstr Bact., 1921, 5, p. 21.

The total number of cases that it was possible to examine during the brief course of the epidemic is too small to warrant definite conclusions. The significant feature is the drop in the general incidence of gram-negative cocci. All forms, including M. catarrhalis, occurred less frequently in influenza cases than in common colds. Curiously enough, the incidence of M. catarrhalis was found to be lower in cases of influenza than in the group of normal persons. It would seem that in these cases at least, the gram-negative forms had been crowded out or overgrown by some more virulent organism, judged by the normal incidence level established in previous studies.

AN ATTEMPT TO DETERMINE THE SPECIFIC RELATIONSHIP OF MICROCOCCUS CATARRHALIS TO COMMON COLDS

Experimental data have been presented which furnish evidence of the presence of M. catarrhalis in a large percentage of common colds and influenza. The same group of organisms, however, has been found in normal persons just as frequently. If any significance was to be attached to the presence of this organism in the pathologic nose and throat, it seemed necessary to investigate certain possibilities. It was necessary to determine, whether in given cases of colds, M. catarrhalis had been present on the mucous membrane as a normal inhabitant previous to the development of the infection. If it were present for some time before the onset of symptoms, did it increase perceptibly n numbers with the developing infection, and thereby exercise a possible symbiotic relationship with the real causative agent of the nflammation? There was also the possibility that certain colds might be encountered which would show no previous carrier condition of M. catarrhalis, but presence of the organism during the infection. In such cases, it was important to determine whether the organism gained entrance into the inflamed area coincidently with developing symptoms, previous to that time, or somewhat later in the course of the infection. If these points could be settled, more exact data regarding the specific relationship of M. catarrhalis would be available.

With these ideas in mind, a group of 10 normal persons was selected, and each one subjected to routine daily cultures over a period of 2½ months. Each subject was watched closely for the onset of symptoms which would indicate an oncoming cold. Following the initial examination, detailed studies of each culture were not made, except when colds developed. The routine procedure consisted of a critical examination of the plates each day, when an estimated percentage was recorded of the number of different types of colonies which had developed. This included the 4 more important groups, the green-producing colonies, the Pfeiffer-like colonies, staphylococcus, and gram-negative-like colonies. From time to time, at intervals of perhaps 2 weeks, detailed studies were made of gram-negative-like colonies in order to maintain a general idea of the specific species comprising that flora.

During the course of these experiments, 5 of the 10 subjects in the series developed one or more colds, the other 5 remaining normal throughout the period of observation.

Gram-negative cocci were isolated from all of the subjects who remained normal, at some time during the course of the experiments. They were present in variable numbers in practically all plate cultures during the series. Only 2 of them, however, subjects A7 and A5, gave cultures of M. catarrhalis while under observation.

Subjects A1, A2, A3, A6 and A9 developed colds at different times during the experiments. Subject A1 showed gram-negative cocci of the Diplococcus crassus type when first examined, with M. catarrhalis absent. Gram-negative-like colonies were not numerous. An acute pharyngitis developed about 2 weeks later, at which time M. catarrhalis was identified and gram-negative-like colonies constituted 45% of total colony development from swabs. Following the cold, catarrhalis-like colonies dropped to about 10 or 15% of all colonies. A little more than a month after the first pharyngitis, a second inflammation and cough were observed. Again catarrhalis-like colonies increased in relative numbers, beginning noticeably the day before symptoms appeared, when they constituted 50% of all colonies. On the day that the cold was apparent, 30% of gram-negative-like colonies were present, the next day 40%, and the second day, 80%. M. catarrhalis was demonstrated at this time and likewise 4 days later. Following the cold, the incidence of gram-negative-like colonies fell to a level of from 10 to 20%, with M. catarrhalis unable to be demonstrated.

The initial examination of subject A2 showed a considerable percentage incidence of gram-negative-like colonies. The organisms were found to belong to chromogenic group 5. On the second day after initiation of study, an acute rhinitis developed. M. catarrhalis could not be demonstrated. Three weeks after the first cold, a second rhinitis was diagnosed. Gram-negative-like colonies were few in number, almost negligible, and these few were the same chromogen 5. On the third day of the infection gram-negative-like colonies had increased in number to 30% of the total number of colonies, and M. catarrhalis was isolated, together with the chromogen. They continued to increase in numbers, and the next day the group represented 50% of the flora.

The history of subject A3 resembles that of A2 as regards incidence of colds. An acute rhinitis developed the second day of observation. Large numbers, 50%, of chromogen 5 organisms were observed early in the infection, and indeed colonies of the gram-negative type dominated the plates. M. catarrhalis was not isolated until 8 days after the beginning of symptoms, and its presence was transitory as it was negative 2 days later and continued to remain so. A second rhinitis developed about 6 weeks later, and although gram-negative-like colonies showed an increase in numbers, M. catarrhalis could not be found.

Subject A6, at the beginning of the study, had a low incidence of gram-negative-like colonies which were of the chromogenic groups. An acute rhinitis developed in 3 weeks, but no marked increase in gram-negative organisms could be observed, and M. catarrhalis was absent. M. catarrhalis was only isolated once during the study of cultures from this subject. Almost a month after the termination of the cold, there was a sudden flare-up of catarrhalis-like colonies with a colony percentage incidence of 85%. M. catarrhalis was identified. This marked increase was transitory, the number decreasing shortly with M. catarrhalis absent in cultures thereafter.

The gram-negative cocci were apparently of little importance in the history of subject A9. They could be identified in most cultures as constituting about 10% of the flora. M. pharyngis siccus and group 5 chromogens were identified. M. catarrhalis was isolated at no time, and although a mild pharyngitis developed 3 weeks after the start of the study, no relative change could be observed in the gram-negative flora.

Analysis of the 5 cases in which colds developed brings out certain facts. A number of colds are encountered with which M. catarrhalis is concerned in no way. Although gram-negative cocci could regularly be isolated during the extended examinations from subjects A3, A6 and A9, they apparently were concerned in no way with the colds which developed. M. catarrhalis was demonstrated in both subjects A3 and A6 but could not logically be connected in any way with the colds which were observed. A particular point of interest observed in subject A6 was the sudden and marked increase in gram-negative forms which occurred without apparent effect on the mucous membrane. M. catarrhalis constituted 85% of all colonies present on plates at that examination. Such transitory domination of the flora by a particular organism seems to demonstrate the frequently changing character of the bacterial flora in at least a considerable proportion of persons. Seemingly, a sudden dominant position in the flora can be attained by several organisms, possibly through invasion of the respiratory mucous membrane by a strain more virulent than its fellows. Such instances have been observed for the pneumococcus, Bacillus mucosus capsulatus and the Pfeiffer bacillus as well as M. catarrhalis, in a study of normal throats over extended periods.

The two cases of rhinitis which occurred in the study of subject A1 influenced certain changes in the gram-negative flora. M. catarrhalis had not been present in cultures previous to the infection. Increased numbers of gram-negative-like colonies were observed the day before symptoms and during the height of the infection. Gram-negative forms, including M. catarrhalis, were distinctly dominant in plate cultures. With subsidence of the infection the organisms tended to disappear. The tremendous increase in numbers may have been due to the acute process so altering the environment that the organisms took hold and grew more rapidly at the seat of the disease. In the rhinitis of subject A2, M. catarrhalis, which was observed late in the cold, can probably best be interpreted as one of the secondary invaders.

Eight colds, then, have developed in these 5 persons. M. catarrhalis was apparently concerned in no way with 5 of them. In one, it seems to have invaded secondarily the previously inflammed area. Two cases may have been caused by M. catarrhalis. It has been emphasized in a previous paper, however, that one cannot ascribe a causative relationship to a given organism, even when the evidence is reasonably favorable, for there is no direct knowledge of the period of incubation of such infections. The presence of M. catarrhalis at the time of infection may have meant merely that under the stimulus of the true exciting agent of the cold, these organisms, normally in the throat, although present in such small numbers that they could not be demonstrated, rapidly began to multiply, and possibly acquired as well a distinct pathogenicity. Thus one cannot determine whether the presence of the organism indicated a direct or merely symbiotic relationship to the infection.

THE EXTENT OF THE CARRIER STATE FOR MICROCOCCUS CATARRHALIS

Organisms of the type of M. catarrhalis have been demonstrated in essentially the same relative number of throats, whether they be normal or pathologic. Such being the case, interest is attached to the question of whether the organism is to be considered as a permanent inhabitant of the throat, with such frequent occurrence, or whether the presence of the organism in a given throat is limited, and its status subject to frequent change.

M. catarrhalis does not appear to be carried in appreciable numbers in throats of convalescents from colds. No general deduction can be made from the few cold cases in the "A" series, in which M. calarrhalis was involved. It was found in these cases, however, that M. catarrhalis was carried a relatively short time following convalescence.

More important, particularly in view of the common incidence of M. catarrhalis, is the relation of the organism to the normal person. Is the organism present for a long period of time in a given throat; is this the usual occurrence in a considerable proportion of normal people; and is it therefore most logically to be considered as a normal throat saprophyte? Or, on the contrary, does it inhabit a given throat for brief periods of time with the probability of frequent transfer from person to person?

Three different groups of normal persons have been studied at different times of the year. Cultures from the individuals of each group were taken at weekly intervals over a period of 2 months, from one group for 2½ months. The first group of 5 persons was studied during the winter months of Nov., Dec. and Jan. No complicating colds developed during the period of observation. Three of the 5 subjects did not show M. catarrhalis. In fact gram-negative-like colonies were in the minority in practically all of the plates. The types of organisms of the gram-negative group which were represented were members of the chromogenic group, with one case showing M. pharyngis siccus.

The fourth subject of the group possessed a throat flora which was consistently dominated by members of the gram-negative group. The percentage of colonies on the various plates, which were representative of gram-negative cocci, varied from 40 to 90%, and the usual finding approached the higher figure. M. catarrhalis was the chief organism among the gram-negative-like colonies, but chromogenic cocci were also isolated.

The flora of the last member of the group, subject 5, was likewise characterized by preponderance of gram-negative-like colonies over other forms. This condition continued with periodic variations throughout the period of observation. The flora consisted of M. catarrhalis as well as a chromogenic coccus of group 5.

A different group of 6 persons was studied in the same way during the Spring months, April, May and early June. Two of them were negative for M. catarrhalis during the experiments, although the familiar chromogens were observed in one. Three subjects, NC 56, NC 154 and NC 505, gave positive cultures of M. catarrhalis throughout the period of observation. The other subject of the series, NC 228, harbored M. catarrhalis in the upper respiratory tract at the initial examination and for one month thereafter, following which cultures proved to be negative.

The third study of normal persons was conducted during the summer months, June, July and Aug. Five persons constituted this group. In cultures from 2 of them, S2 and S5, M. catarrhalis could not be found at any time. Chromogens were present in one case, M. pharyngis siccus in the other, while the latter likewise gave positive cultures of the meningococcus for 3 successive weeks, after which that organism disappeared. The other 3 subjects, S1, S3 and S4 all showed consistently cultures of M. catarrhalis. Subjects S3 and S4 gave positive cultures throughout the period of observation. Cultures from subject S1 failed to reveal the presence of M. catarrhalis at the initial examination but were positive thereafter until the work was terminated.

These separate groups of persons, studied during different seasons of the year, have given results which furnish rather conclusive evidence of the role played by the group of gram-negative cocci, and particularly by M. catarrhalis. The case incidence of the catarrhalis group, considering the various series of persons as a unit, corresponds to the same general percentage observed in the larger series of normal persons on whom a single examination was made. All subjects who showed M. catarrhalis in throat cultures were, with a single exception, carriers of that organism throughout the various periods of observation. The one exception was positive at the beginning of the study but became negative for M. catarrhalis after one month. The previous length of the carrier state was of course indeterminate. One feels that this group of organisms, M. catarrhalis, constitutes a species which has become highly acclimatized to the mucous membrane of the upper respiratory tract. A large proportion of persons carry it in the throat for long periods of time without visible effect on the lining membrane. Rare instances may arise in which a more virulent strain may be involved in respiratory infection. In general it would seem to lead a saprophytic existence in numerous throats, a condition comparable to that of the commonly recognized saprophytes of the mouth.

COMPARATIVE VIRULENCE OF STRAINS OF MICROCOCCUS CATARRHALIS FROM NORMAL SOURCES AND FROM COLDS

M. catarrhalis has been demonstrated to be present in normal persons and in cases of colds with about equal frequency. In persons during influenzal infections it has been found less frequently than in normal persons. If any pathogenic properties are to be conceded the organism, it would seem essential to establish, among other things, a relatively heightened virulence of the coccus, when found in cases of colds and influenza.

Various strains have been tested in regard to their virulence for mice. These strains have been derived from different types of respiratory infection and from normal persons. While no definite information was at hand, it has been assumed that M. catarrhalis possesses the characteristic, common to so many bacteria, of a decline in virulence following continued cultivation on artificial medium. The following uniform procedure has been followed, in order that virulence might be tested as soon as possible after recovery of the strain from the throat, and likewise that directly comparable data might be obtained. Colonies were picked from blood-agar plates to blood-agar slants. Purity of culture was established by morphologic examination. Transfers were then made to dextrose medium, and if fermentation was not produced in 24 hours, a transfer was made from the original blood-agar slant to a second tube of the same medium. This 24-hour old culture was employed for the virulence tests. It was consequently in its third generation on blood agar. By this method conditions were exactly comparable in all instances. Classification of the organism was verified later by the usual methods.

In preparing cultures for inoculation, care was taken to seed as nearly as possible the same extent of blood-agar medium. Growths were washed off in 2 c c of warm sterile broth and mice were inoculated intraperitoneally at once. In order to obtain an idea of the usual lethal dose for mice, preliminary tests were made with 4 strains from cases of colds. Mice were inoculated, respectively, with one-eighth, one-fourth, and one-half of the slant culture of the first strain. None of the mice died within 6 days. The next 3 cold strains were tested by inoculating one-eighth, one-fourth, one-half and one slant into a series of mice. Death occurred in 24 hours in all 3 animals receiving the massive doses of 1 whole slant. One animal injected with one-half slant died in 3 days, strain C557J. All others survived. It was felt that the use of such tremendous doses as one full slant, with an attendant volume of 2 c c did not constitute a test of any value when inoculated into so small an animal as the white mouse. In subsequent determinations, each strain was injected in doses of one-fourth and one-half slant. Eight normal strains have been tested and 8 cold strains. Death was produced by 2 of the normal strains, S3D and 505A2, in one-half agar slant doses. All other animals survived. Three of the cold strains produced death, C629D influenza, C557J rhinitis and bronchitis, and C82H16 rhinitis, at periods of 4 days, 3 days, and 2 days, respectively. The lethal dose of strains C629D and C557J was one-half slant, of strain C82H16 one-fourth slant. The larger dose of this strain, curiously enough, did not kill the animal. Profound symptoms of toxemia were evidenced in all of the animals receiving the larger dose and usually in those receiving the smaller. Necropsy cultures from the mice which succumbed failed to reveal the organism in the heart blood. Three cases C557J, C82H16 and S5D, gave cultures from the peritoneal fluid, C557J from the pleural fluid as well. The only pathologic changes which could be observed were a variable increase in the amount of peritoneal fluid, enlargement and hyperemia of the spleen, occasional subserous hemorrhage in the intestines and in one case increased pleural fluid. There were no indications of a generalized infection, pathologically or bacteriologically.

The organism possesses little or no virulence for mice. Tremendous doses were required to produce death which was evidently due more to the toxic effect of the bacterial protein than any growth and reproduction of the organism in the tissues. Furthermore, no difference could be determined between the lethal effects of strains isolated from normal and from cold sources.

Various strains studied were practically avirulent for rabbits. No definite experiments were conducted to determine the degree of virulence, but in immunizing animals against numerous strains, doses of one-fourth blood-agar slant of live organisms were commonly employed, intravenously as the initial dose, followed by one-half and one whole slant on the next two days, without ill effect.

DISCUSSION

Cocci which fail to retain the Gram stain constitute one of the more common groups of bacteria which may be found in the upper respiratory tract of man in both health and disease. They can be divided on the basis of fermentative power and growth characteristics, into several different groups. The largest group encountered in this study is made up of those organisms which fail to produce acid through action on any of the more common carbohydrates. These organisms have been recognized as constituting a single group, M. catarrhalis which, however, may be divided into 5 subgroups on the basis of cultural differences. A second group of organisms, characterized by the production of a greenish yellow pigment, occurs in the human respiratory tract with about the same frequency as the first group. These strains likewise may be divided into 6 different subgroups on the basis of differences in fermentation. The meningococcus of Weichselbaum, Diplococcus crassus Kral, and M. pharyngis siccus complete the list of gram-negative cocci found.

The essential purpose of this study was to investigate the relationship which these gram-negative cocci bear toward upper respiratory tract infection. Certain data have been obtained. Some member of the general group can be found in three-fourths or more of all cases, M. catarrhalis in about half that number. The percentage of incidence of all groups is, however, essentially the same in normal as in pathologic throats. In fact, they are less frequent in persons with influenza than in normal persons, an indication that they have been crowded out by a more virulent species. No single group of gram-negative cocci, other than M. catarrhalis, occurs with special frequency in common colds and influenza. The variable and irregular occurrence of these other groups would seem to limit any importance possessed by gram-negative cocci to the rôle played by M. catarrhalis.

Extended study of certain normal persons found to harbor M. catarrhalis in the throat has led to the conclusion that the organism is decidedly a permanent inhabitant of the throat, rather than a temporary invader. It lodges in a considerable percentage of throats, becomes readily acclimatized to that environment, and lives there for long periods of time, leading apparently a saprophytic existence. Finally, various strains from normal sources and from colds have been found to be avirulent for white mice and rabbits. Lethal effects have been obtained with a few strains, but only when massive doses were employed. Strains from normal persons and from cases of common colds present, moreover, no distinguishable differences in lethal power.

Rarely, circumstances occur in which it may be possible to place some emphasis on M. catarrhalis in explaining the pathogenesis of a given cold. Such an instance has been discussed in connection with the two rhinites observed in subject 1 of the "A" series.

Observation of M. catarrhalis in normal throats and in various respiratory infections, justifies but one conclusion. The organism is only rarely involved in the pathogenesis of acute infections of the upper respiratory tract in man. Based on its like incidence in colds and in normal persons, the actual decline in incidence in influenza, its long contained existence on the mucous membranes of many normal persons, and finally its lack of virulence, irrespective of origin from normal or patholotic throats, the logical deduction is that its usual sphere is that of a harmless saprophyte.

CONCLUSIONS

Gram-negative cocci which occur in the nose and throat normally and in acute upper respiratory infections may be grouped according to cultural characteristics and fermentative differences. Such a classification is presented in table 1 and the succeeding discussion.

No essential difference was distinguished between the incidence of the various groups of gram-negative cocci in common colds, and in a like series of normal persons. In epidemic influenza, the incidence was less than in normal persons.

M. catarrhalis, the most common member of the group, is carried for long periods of time in the throats of many normal persons, constituting a permanent member of the normal throat flora.

No distinguishable differences in virulence for mice and rabbits could be determined between strains of M. catarrhalis from normal sources and those from colds or influenza.

These observations do not indicate that Micrococcus catarrhalis is generally concerned in the pathogenesis of common colds or influenza.